Bioactive triterpenoids from the caffeine-rich plants guayusa and maté

G. Chianese, S.D. Golin-Pacheco, O. Taglialatela-Scafati, J.A. Collado, E. Munoz, G. Appendino, F. Pollastro

PII: S0963-9969(18)30792-0
DOI: doi:10.1016/j.foodres.2018.10.005
Reference: FRIN 7975

To appear in: *Food Research International*

Received date: 31 May 2018
Revised date: 17 September 2018
Accepted date: 1 October 2018

Please cite this article as: G. Chianese, S.D. Golin-Pacheco, O. Taglialatela-Scafati, J.A. Collado, E. Munoz, G. Appendino, F. Pollastro , Bioactive triterpenoids from the caffeine-rich plants guayusa and maté. Frin (2018), doi:10.1016/j.foodres.2018.10.005

Bioactive triterpenoids from the caffeine-rich plants guayusa and maté

G. Chianese[a], S. D. Golin-Pacheco[b], O. Taglialatela-Scafati[a]*, J. A. Collado[c,d,e], E. Munoz[c,d,e], G. Appendino[f], F. Pollastro[f]*

[a]*University of Naples Federico II, Department of Pharmacy, School of Medicine and Surgery, Naples, Italy*

[b]*Federal University of Paraná, PR, Brazil*

[c]*Instituto Maimónides de Investigación Biomédica de Córdoba (IMIBIC), Córdoba, Spain.*

[d]*Departamento de Biología Celular, Fisiología e Inmunología, Universidad de Córdoba, Córdoba, Spain*

[e]*Hospital Universitario Reina Sofía, Córdoba, Spain*

[f]*Department of Pharmaceutical Sciences, University of Eastern Piedmont, Novara, Italy*

*Corresponding authors:

Orazio Taglialatela-Scafati, University of Naples Federico II, Department of Pharmacy School of Medicine and Surgery, Naples, Italy; phone: +39 081678509; Fax: +39 081678552; Email: scatagli@unina.it

Federica Pollastro, Department of Pharmaceutical Sciences, University of Eastern Piedmont, Novara, Italy: phone: +39 0321375844; Fax: +39 0321375621; Email: federica.pollastro@uniupo.it

Abstract

Unlike all other caffeinated plants, guayusa (*Ilex guayusa* Loes.) and maté (*Ilex paraguariensis* A. St. Hill) contain high amounts of pentacyclic triterpenoid acids and alcohols. A phytochemical investigation on these plants revealed a similar triterpenoid profile and a content of ursolic acid (0.7-1%) and amyrin esters (up to 0.5%), quite unusual for dietary plants. The major constituent of the amyrin complex from both plants is α-amyrin palmitate (**2a**), accompanied by lower amounts of its corresponding palmitoleate (**2b**) and by the corresponding constitutional isomers from the β-series (**3a** and **3b**, respectively). Ursolic acid (**1**) was identified as the responsible for the activity of maté and guayusa extracts in the activation of TGR5, a nuclear receptor of relevance for the prevention and management of diabetes and metabolic syndrome because of its involvement in the regulation of energy expenditure and insulin sensitivity.

Keywords: *Ilex guayusa*, *Ilex paraguariensis*, TGR5, metabolic syndrome, ursolic acid

1. Introduction

Guayusa *(Ilex guayusa* Loes.), an Amazonian rainforest plant belonging to the family Aquifoliacee, has a rich history of human consumption for ritual purposes as well as a beverage (Dueñas et al., 2016; Garcia-Ruiz et al., 2017). Archaeological findings of guayusa leaves have been identified in several shamanic tombs dating as early as 500 BC, and the consumption of guayusa is documented in traditional societies of the Northern Andean region, from Colombia to Peru (Dueñas et al., 2016). In these populations, guayusa is not only a convivial beverage associated to beneficial health properties, but also a ritual plant often consumed before, during, or after ingesting the hallucinogenic brew ayahuasca to “wipe out its bitter taste” and “prevent hangover” (Schultes and Raffauf, 1990). The use of guayusa-aromatized alcohol beverages is also documented all over the Northern Andean region (Dueñas et al., 2016). *I. guayusa* is related to *Ilex paraguariensis* A. St. Hill, commonly known as “yerba mate” or “maté” (Loizeau et al., 2005), a plant that enjoys a "national infusion” status in Argentina (Bracesco et al., 2011). However, extracts from the two plants are prepared in different manners; thus, guayusa is consumed as a brewed tea, while maté is prepared as a decoction in a gourd recipient, from which it is sucked with a drinking straw called *bombilla* (Bracesco et al., 2011). Diversities also exist in terms of the diffusion of the two herbal beverages. An estimated 30% of the population in South America has been estimated to drink maté daily, and, with a production of more than 1,400 metric tons/year, the plant is exported worldwide (Gnoatto et al., 2005). Conversely, guayusa has long remained a local product, only available in the producing countries, and especially Ecuador, and no data are available on its production volumes. The recent introduction into the US market of guayusa-based products (Runa® tea and soft drinks) has, however, the potential to substantially expand the consumption of this plant. Guayusa is often associated to other stimulant herbs (green tea, coffee, guarana) in products presented as natural alternative to energy drinks, and is claimed to maintain

wakefulness as well as induce a pleasant tranquillity, since the effects of caffeine would be moderated by an high concentration of theanine (http://www.medicinehunter.com/guayusa), a claim unsubstantiated. Overall, guayusa is presented as a “healthier” version of maté, with anti-oxidant properties similar to those of green tea. (Pardau et al. 2017).

Apart from the doubtful claim that guayusa represents the richest source of caffeine known (over 7.5%) (Raetsch, 2005) and contains a high concentration of theanine ((http://www.medicinehunter.com/guayusa), little phytochemical information is available on this plant. This paucity of data, the growing popularity of guayusa, and the discovery in our preliminary tests that guayusa extracts potently activate membrane receptor G-protein-coupled bile acid receptor 5 (TGR5) combined to provide a rationale to identify its active constituents, to investigate its phytochemical profile, and to compare it with the one of maté. The activity of guayusa extracts on TGR5 was especially interesting, since this G-protein coupled receptor is critically involved in the regulation of basal metabolism and energy expenditure (Duboc et al. 2014), and there is growing awareness that its modulation has beneficial health effects also on inflammation and immunity (Guo et al. 2016; Perino and Schoonjans 2015; Kuipers et al., 2014).

2. Materials and methods

2.1 Materials

Silica gel 60 (70-230 mesh), aluminium oxide neutral 90 – Activity 1 and Merck 60 F254 (0.25 mm) TLC plates were purchased by Merck (Germany). Ethyl acetate, petroleum ether, methanol, ethanol, ethyl ether, DMSO, formic acid, H_2SO_4 and reagents were purchased form Sigma-Aldrich (Italy). ^{1}H (400 MHz) and ^{13}C (125 MHz) NMR spectra were measured on a Varian INOVA spectrometer. Chemical shift was referenced to the residual solvent signal ($CDCl_3$: δ_H 7.26, δ_C 77.0). Homonuclear ^{1}H connectivities were determined

by a COSY experiment. GloMax 96 microplate luminometer was purchased by Promega (Madison, WI, USA). A gas-chromatograph Agilent Technologies 6850 Series II/5973 Network MS D system coupled with a Mass Selective Detector Agilent Technologies 5973 Network was used. The carrier gas was helium, purity 99.999%, at 0.8 mL/min, HP-5MS capillary column (Agilent, 5% Phenyl Methyl Siloxane) (30m × 0.25mm i.d., 0.25 µm film thickness). Column temperature was initially kept at 150 °C for 15 min, then gradually increased to 300 °C at 5 °C/min rate and finally held at this temperature for 10 min. Temperature of transfer line was at 280 °C. The software MSDchem (Agilent Technologies) has been used to analyze data. Commercial samples of guayusa were purchased from Ecuadorian Rainforest LLC (ER, 222 Getty Ave - Clifton, NJ, USA), and those of maté from Rei Verde (Iléopolis, RS, Brazil). All samples were made up by dried powdered leaves. Voucher specimens of the samples are kept at the Novara laboratories. All triterpenoids were identified by comparison with authentic samples available from previous studies (Del Prete et al., 2017), or through the TriForC Consortium (Goossens et al., 2018). For the spectroscopic data of all known compounds identified, see: https://nmrpredict.orc.univie.ac.at/similar/eval.php .

2.2 Extract screening

Samples (30 g) of powdered leaves of *I. guayusa* and *I. paraguariensis* were extracted under stirring with 300 mL acetone for 1 h, affording, after solvent evaporation, 2.33 g (7.8%) and 2.36 g (7.9%), respectively, of a dark green syrup, positive in the TGR5 assay (EC_{50} <10 µg/mL, see section 2.6). The extracts were dissolved into the minimal amount of methanol, and then 7 g of silica gel was added (ratio extract /silica 1:1 w/w) and the suspensions evaporated. The powders obtained in this way were stratified on a layer of Celite (7 g) and fractionated by vacuum filtration with 70 mL of, respectively, petroleum ether, ethyl acetate and tetrahydrofuran. In this way, a low-polarity sub-fraction (A,

petroleum ether), a medium polarity sub-fraction (B, ethyl acetate), and a high polarity fraction (C, tetrahydrofuran) were obtained. Yields were as follows: guayusa: 700 mg (2.3%) of A, 760 mg (2.5%) of B; 640 mg (2.2%) of C. Maté: 708 mg of A (2.4%), 1.0 g (3.3%) of B and 652 mg (2.2%) of C. Only fraction B was active in TGR5 assays (EC_{50} < 10 μg/mL, see section 2.6), and its ^{1}HNMR analysis showed the presence of ursolic acid and an amyrin complex as major constituents.

2.3. Isolation of triterpenoids from guayusa (Ilex guayusa Loes*.).*

A 450 g sample of guayusa powder was extracted with acetone (2 × 4.5 L) in a vertical percolator at room temperature, affording, after solvent evaporation, 39.2 g (8.72%) of a dark green syrup, that was taken up in 390 mL of acetone (ratio extract/solvent 1:10 weight/volume) at 70 °C. After complete solution, the extract was maintained at room temperature for 1 h and then cooled at - 8 °C for 12 h to obtain a copious precipitate of ursolic acid (**1**) containing ca 10% oleanolic acid by ^{1}H NMR analysis. The mother liquors were mixed up with silica gel (32 g) and evaporated to a powder that was fractionated by gravity column chromatography on neutral alumina (80 g) with a petroleum ether-EtOAc gradient as eluent (from 9:1 to 2:10). Fractions were combined according to their TLC profile, and the least polar one (7.7 g) was re-fractionated by column chromatography on silica gel (300 mL), with a petroleum ether/EtOAc gradient (from 98:2 to 70:30; 80 mL fraction). Fraction I contained only lipids, and was discarded. Fraction II (1.43 g) was a crude mixture of amyrin esters, further purified by column chromatography on silica gel (150 mL, petroleum ether/EtOAc gradient, from 100% petrol-ether to 98:2 petroleum ether-EtOAc, 10 mL fractions) to afford an amyrin ester complex (480 mg) composed by α-amyrin palmitate and palmitoleate (**2a** and **2b**) and their corresponding isomers from the β-series (**3a** and **3b**, respectively). Fraction III (307 mg) and fraction V (533 mg) were both purified by Biotage flash chromatographer (Isolera One model) with 12 g RP C-18

cartridge and solvent A: methanol - 0,03% formic acid, solvent B: water - 0,03% formic acid and gradient form 70:30 (A:B) to 100% B to afford respectively 69 mg of uvaol aldehyde (**5**) and 170 mg of uvaol (**4**).

2.4. Isolation of triterpenoids from mate (Ilex paraguariensis A. St. Hill).

A sample (2 Kg) of maté was extracted with petroleum ether (2 × 8.5 L) in a vertical percolator at room temperature affording 46.2 g (2.3%) of a dark yellow syrup of amyrin esters. The defatted plant material was extracted with acetone (2 x 6.5 L) in a vertical percolator at room temperature, affording 80 g (3.73%) of dark green paste. The latter was dissolved in refluxing acetone (800 mL) and then worked up as described for guayusa to remove ursolic acid (**1**) by precipitation (15.31 g, 0.71%) and obtain the amyrin complex by gravity column chromatography, whose yield was higher than from guayusa (10.7 g, ca 0.5%). Other samples of mate, even from the same producer, had a similar contents of ursolic acid, but a lower contents of the amyrin complex (ca. 0.1-0.2%).

2.5. Semisynthesis of α-amyrin palmitate

A sample of petroleum ether extract from maté (46.25 g) was dissolved in 5% KOH in methanol (500 mL) and refluxed for 2 h. The reaction was worked up by dilution with brine (200 mL), acidification with 2N H_2SO_4, and partial evaporation. Extraction with petroleum ether afforded a yellow syrup (30 g), next purified by GCC [300 mL silica gel, petroleum ether-EtOAc gradient (from 100% to 70:30) as eluant, 80 mL fraction] affording 24.5 g of a crude mixture of amyrins, a portion of which (1.45 g) was dissolved in DMSO (100 mL) and oxidized with SIBX (45%, 5.9 g) at 55 °C. After 15 minutes, the reaction in worked up by dilution with sat. $NaHCO_3$ (50 mL) and brine (20 mL), and extracted with petroleum ether-ethyl ether 3:1 (3 x 20mL). After drying (Na_2SO_4), filtration and evaporation, a crude mixture of amyrinones (1.33 g) was obtained. The latter was crystallized from methanol to

afford 560 mg of α-amirinone **5** as a white powder. A portion of this (75 mg) was dissolved in dry THF (8 mL) and treated with an excess of $LiAlH_4$ (209 mg) The reaction was stirred at room temperature and next quenched with 2 *N* H_2SO_4 and extracted with EtOAc (3 x 10 mL). The organic phase was washed with sat. $NaHCO_3$, dried (Na_2SO_4), filtered and evaporated to afford 49.0 mg (68%) α-amyrin (**2**). A 100 mg sample of the latter was dissolved in CH_2Cl_2 (15 mL), and treated with palmitic acid (122 mg, 2 mol. equiv.) and EDC (91 mg, 2 mol. equiv.) and DMAP (57,9 mg, 2 mol. equiv.). After refluxing for 4 h, the reaction was worked up by dilution with sat. $NaHCO_3$ (5 mL) and brine (5 mL), and next extracted with CH_2Cl_2 (3x10 mL). After drying ($Na_2SO_{4)}$ and evaporation, the residue was purifgied by GCC on silica gel (petroleum ether as eluant) to afford 144 mg (96%) of α-amyrin palmitate **2a**.

2.6. TGR5 assay

Chinese hamster ovary (CHO) cells were obtained from ATCC (Manassas, VA) and were maintained in DMEM supplemented with 10% (V/V) fetal bovine serum (FBS) and antibiotics. For the TGR5 assay, a stable cell line was generated by transfection of CHO cells with 3 μg of TGR5 expression plasmid (pCMVSPORT6/TGR5), 3 μg of cAMP response element (CRE)-driven luciferase reporter plasmid (pCRE-Luc), and 0.5 μg of neomycin-resistant gene expression plasmid (pcDNA3.1 (+)) using Lipofectamine 2000 reagent (Fisher-Scientific, Madrid, Spain). The transfected cells were selected with 100 μg/mL G418 sulfate, and single clones were grown in a 96-well plate to select the CHO-TGR5-CRE-Luc cell line used to functional assays. TGR5-expressing CHO cells were treated with increasing concentrations of ursolic acid or plant extracts, followed by luciferase assays. Litocholic acid (LCA) used as an internal control to predict efficacy. Cells ($1x10^4$) were seeded the day before the assay on 96-well plate, and then the cells were treated with 10 μM lithocholic acid (LCA) (internal control), or with increasing

concentrations of ursolic acid or plant extracts for 6 h, washed twice with PBS and lysed in 50µl lysis buffer containing 25 mM Tris-phosphate (pH 7.8), 8 mM $MgCl_2$, 1 mM DTT, 1% Triton X-100, and 7% glycerol during 15 min at RT in a horizontal shaker. Luciferase activity was measured using a GloMax 96 microplate luminometer following the instructions of the luciferase assay kit (Promega, Madrid, Spain). All the experiments were performed in triplicate and the mean ± SD is shown.

3. Results and discussion

The G-protein coupled receptor TGR5 (GP-BAR1), a receptor specific for bile acids, regulates glucose homeostasis, lipid metabolism, and energy expenditure, and its activation is therefore of relevance to identify potentially new anti-diabetic agents (Ma and Patti, 2014). As part of a screening of extracts from dietary plants for the activation of TGR5, a crude acetone extracts from guayusa turned out positive in the assay (EC_{50} < 10 µg/mL) (Table 1), and similar results were obtained from maté.

Table 1. Effects on the TGR5 activity of plant extracts and pure triterpenoids

Compound	EC_{50}	Efficacy
Litocholic acid (µM)	10.7 ± 2.3	100
Ursolic acid (µM)	4.7 ± 1.3	125
α-Amyrin palmitate	>50	0
Guayusa crude extract (µg/mL)	7.3 ± 2.1	117
Maté crude extract (µg/mL)	7.9 ± 2.1	119
Guayusa EtOAc extract (µg/mL)	5.9 ± 3.3	113
Maté EtOAc extract (µg/mL)	9.1 ± 3.1	109

Plants from the genus *Ilex* are prolific producers of secondary metabolites that include, apart from xanthines, chlorogenic acid derivatives, flavonoids, triterpenoids, and saponins

(Cardozo Jr. et al., 2016; Pardau et al., 2017). To identify the active principle(s) of the crude extracts, fractionation by solid/liquid partition was used to sort the primary acetone extracts into three sub-fractions with different polarity (petroleum ether, ethyl acetate and tetrahydrofuran sub-fractions) (Scheme 1).

((Insert Scheme 1))

With both extracts, the medium-polarity (ethyl acetate) sub-fraction turned out to be the only active in the TGR5 assay, substantially capturing the whole activity of the crude primary extract. When analysed by ^{1}H NMR, the active fraction was a mixure of pentacyclic triterpenoids, dominated by ursolic acid and by an amyrin ester complex (Figure 1). The latter was also the major constituents of the inactive and less polar petroleum ether sub-fraction.

((Insert Figure 1))

Pentacyclic triterpenoids are typical constituents of plants from the genus *Ilex* and these triterpenes can be viewed as the major non-nitrogenous constituents of the guayusa and maté extract. Indeed, large amounts of ursolic acid (**1**, *ca*. 1%) and amyrin esters (**2a** and **2b** as the main components, ca 0.2%) (Figure 2) were obtained by a combination of direct crystallization and gravity column chromatography.

((Insert Figure 2))

Ursolic acid was contaminated by ca 10% oleanolic acid, that could be removed by re-crystallization from methanol. On the contrary, the amyrin complex could not be

resolved satisfactorily by chromatography or crystallization and was deconvoluted by a combination of spectroscopic and analytical methods. The 4:1 ratio between α- and β-amyrins was established by ^{1}H NMR, after saponification, capitalizing on the different chemical shift of the olefin H-11 proton. The nature of the acylating groups was established by GC-MS analysis of the ethyl esters obtained as products of the transesterification of the amyrin complex (see below). Finally, minor amounts of compounds with a C-18 intermediate oxidation state between ursolic acid (-COOH) and amyrins (-CH_3) were also obtained (uvaol **4** and its corresponding aldehyde **5**) and identified by comparison of their spectral data with those reported in the literature (Liao et al., 2014).

((Insert Scheme 2))

A pure sample of α-amyrin palmitate, the major constituent of the amyrin complex, was prepared for evaluation in pharmacological tests and to serve as a reference for further studies, following the semi-synthetic procedure illustrated in Scheme 2. Thus, transesterification of the mixture provided, along with an amyrin mixture, a fatty ester fraction dominated by methyl palmitate, with minor amounts of its corresponding palmitoleate. Attempt to resolve the α/β-amyrin mixture by fractionate crystallization of the acetates, a procedure reported in the old literature on these compounds (Simonsen and Ross, 1957), was not fully successful. Conversely, fractionate crystallization of the 3-dehydroderivatives provided a pure sample of 3-dehydro-α-amyrin (**6**), next quantitatively reduced to α-amyrin (**2**) with $LiAlH_4$. Palmitoylation by Steglich esterification (palmitic acid, DCC, DMAP) was then uneventful and afforded a pure sample of (**2a**) as a colorless oil in overall 44% yield from the crude amyrin mixture.

The triterpenoid profile of maté was similar to the one of guayusa, and pentacyclic triterpenoids do not qualify therefore as suitable markers to discriminate between the two

plants. Large oscillations in the contents of the amyrin fraction (0.2-0.5 %) were, however, observed between different maté samples. Since only a sample a guayusa was available, it was not possible to confirm if similar variations also occur in this plant. The presence of large amounts of non-glycosylated triterpenoids (ursolic acid and amyrin esters) in maté had been already reported in a 1940 article (Mendive, 1940) but, surprisingly, this information was overlooked in later literature on this plant that only mentioned the occurrence of triterpenoid glycosides, while the role of non-glycosidic pentacyclic triterpenoids in the activity of maté is completely neglected. Maté contains bis-desmosidic saponins (matesaponins) based on oleanolic and ursolic acid. While the hydrolysis of ester-linked sugars is relatively easy, the removal of glycosidically-bound sugars requires prolonged refluxing in acids (Gnoatto et al., 2005). It is therefore unlikely that dramatic changes in the ratio between free triterpenoids and their sugar-decorated versions could take place spontaneously during plant storage or preparation of the infusion. On the other hand, leaves of maté and guayusa could retain residual enzymatic activity, and this could affect the ratio between free- and glycosylated triterpenoids.

There is a considerable interest for the beneficial properties of maté and guayusa in the prevention of obesity-related biochemical parameters and in the modulation of various end-points of relevance for inflammation (Kang et al., 2012; Kim et al., 2015). Thus, both pre-clinical and clinical evidence exist that maté can exert beneficial effects on glucose control and blood lipids (Klein et al., 2011; Balzan et al., 2013), and its consumption has been suggested as preventive or even as therapeutic agent for a number of conditions related to metabolic syndrome and cardiovascular disease. However, the nature of the active ingredient(s) is still unclear. Saponins and chlorogenic acid derivatives were generally considered as responsible for these activities (Bains and Gugliucci, 2017), but our finding of large amounts of triterpenoid acids suggest further candidates for activity. Thus, by activating the TGR5 receptor, ursolic acid increases incretin secretion and

reduces blood glucose level (Lo et al., 2017), and, by inhibiting protein tyrosine phosphatase 1B (PTP-1B), it supresses a negative regulator of the insulin-signaling pathway (Guzman-Avila et al., 2018). On the other hand, amyrins are known to be selective inhibitors of the enzymatic degradation of the endocannabinoids 2-arachidonoyl glycerol (2-AG) (Chicca et al., 2012), a powerful insulin-sensitizer and glucose uptake booster (Chanda et al., 2017).

Among the triterpenoids isolated from the bioactive fraction of maté and guayusa, only ursolic acid potently activated TGR5 with an EC_{50} = 4.7 μM. This potency level has already been reported for ursolic acid (Lo et al., 2017) as well as for other pentacyclic triterpenoid acids (Genet et al., 2010), but our knowledge of the structure-activity relationship of this class of compounds are, overall, still preliminary. Conversely, the amyrin esters fraction, as well as pure α-amyrin palmitoleate **2a,** were inactive (EC_{50} > 50 μM), an observation that suggest a critical role for the carboxylate group.

4. Conclusions

In this work, we have analysed the triterpenoid fraction of mate and guayusa, popular not only as caffeine-rich beverages, but, similarly to tea, cocoa and coffee, as nutraceuticals. The isolation of large amounts of pentacyclic triterpenoids from this plant and from guayusa suggests additional active principles and mechanisms of action. The presence of high concentrations of triterpenoids is also a distinctive trait of maté and guayusa compared to other caffeinated beverages, and provides a molecular rationale for investigating the beneficial properties of these beverages in controlled human clinical trials.

Acknowledgements

NMR spectra were recorded at “Centro di Servizio Interdipartimentale di Analisi Strumentale”, Università di Napoli Federico II. The research leading to these results has received funding to TriForC from the European Community's Seventh Framework Programme [FP7/2007-2013] under grant agreement 613692, A Pipeline for the Discovery, Sustainable Production and Commercial Utilization of Known and Novel High-value Triterpenes with New or Superior Biological Activities.

Conflict of interest

The authors declare that they have no conflict of interest

References

Bains, Y., Gugliucci, A. (2017). *Ilex paraguariensis* and its main component chlorogenic acid inhibit fructose formation of advanced glycation endproducts with amino acids at conditions compatible with those in the digestive system. *Fitoterapia* 117, 6-10.

Balzan, S., Hernandes, A., Reichert, C. L., Donaduzzi, C., Pires, V. A., Gasparotto Jr, A., Cardozo Jr, E. L. (2013). Lipid-lowering effects of standardized extracts of *Ilex paraguariensis* in high-fat-diet rats. *Fitoterapia*, 86, 115-122.

Bracesco, N., Sanchez, A. G., Contreras, V., Menini, T., Gugliucci, A. (2011). Recent advances on *Ilex paraguariensis* research: minireview, *J Ethnopharmacol* 136, 378–384.

Cardozo Jr., E. L., Morand, C. (2016). Interest of mate (*Ilex paraguariensis* A. St.-Hil.) as a new natural functional food to preserve human cardiovascular health – A review, *J Funct Food* 21, 440–454.

Chanda, D., Oligschlaeger, Y., Geraets, I., Liu, Y., Zhu, X., Li, J., Nabben, M., Coumans, W., Luiken, J. J. F. P., Glatz, J. F. C., Neumann, D. (2017). 2-Arachidonoylglycerol

ameliorates inflammatory stress-induced insulin resistance in cardiomyocytes. *Biol Chem.* 28, 7105-7114.

Chicca, A., Marazzi, J., Gertsch, J. (2012). The antinociceptive triterpene β-amyrin inhibits 2-arachidonoylglycerol (2-AG) hydrolysis without directly targeting cannabinoid receptors. *Br J Pharmacol.* 167, 1596-1608.

Del Prete, D., Taglialatela-Scafati, O., Minassi, A., Sirignano, C., Cruz, C., Bellido, M. L., Muñoz, E., Appendino, G. (2017) Electrophilic triterpenoid enones: a comparative thiol-trapping and bioactivity study*. J Nat Prod.* 80, 2276-228

Duboc, H., Taché, Y., Hofmann, A. F. (2014). The bile acid TGR5 membrane receptor: from basic research to clinical application. *Digest Liv Dis.* 46, 302-312.

Dueñas, J. F., Jarrett, C., Cummins, I., Logan–Hines, E. (2016) Amazonian guayusa (*Ilex guayusa* Loes.): a historical and ethnobotanical overview, *Econ. Bot.* 70, 85–91.

García-Ruiz, A., Baenas, N., Benítez-González, A. M., Stinco, C. M., Meléndez-Martínez, A. J., Moreno, D. A., Rualesa, J. (2017). Guayusa (*Ilex guayusa* L.) new tea: phenolic and carotenoid composition and antioxidant capacity, *J Sci Food Agric* 97, 3929–3936.

Genet, C., Strehle, A., Schmidt, C., Boudjelal, G., Lobstein, A., Schoonjans, K., Souchet, M., Auwerx, J., Saladin, R., Wagner, A. (2010) Structure-activity relationship study of betulinic acid, a novel and selective TGR5 agonist, and its synthetic derivatives: potential impact in diabetes. *J Med Chem.* 53, 178-90)

Gnoatto, S. C. B., Schenkel, E. P., Bassani, V. L. (2005) HPLC method to assay total saponins in *Ilex paraguariensis* aqueous extract. *J. Braz. Chem. Soc.* 16, 723-726.

Goossens, A., Osbourn, A., Michoux, F., Bak, S. (2018) Triterpene messages from the EU-FP7 Project TriForC. *Trends Plant Sci.* 23, 273-276

Guo, C., Chen, W. D., Wang, Y. D. (2016). TGR5, not only a metabolic regulator. *Front Physiol.* 7, e646.

Guzmán-Ávila, R., Flores-Morales, V., Paoli, P., Camici, G., Ramírez-Espinosa, J. J., Cerón-Romero, L., Navarrete-Vázquez, G., Hidalgo-Figueroa, S., Rios, M. Y., Villalobos-Molina, R., Estrada-Soto, S. (2018). Ursolic acid derivatives as potential antidiabetic agents: In vitro, in vivo, and in silico studies. *Drug Dev Res*. 79, 70-78.

Kang, Y. R., Lee, H. Y., Kim, J. H., Moon, D. I., Seo, M. Y., Park, S. H., Choi, K. H., Kim, C. R., Kim, S. H., Oh, J. H., Cho, S. W., Kim, S. Y., Kim, M. G., Chae, S. W., Kim, O., Oh, H.G. (2012). Anti-obesity and anti-diabetic effects of Yerba Mate (*Ilex paraguariensis*) in C57BL/6J mice fed a high-fat diet. *Lab Anim Res* 28, 23-29.

Kim, S. Y., Oh, M. R., Kim, M. G., Chae, H. J., Chae S. W. (2015). Anti-obesity effects of Yerba Mate (*Ilex Paraguariensis*): a randomized, double-blind, placebo-controlled clinical trial, *BMC Complement Altern Med* 15, 338-346.

Klein, G. A., Stefanuto, A., Boaventura, B. C., de Morais, E. C., Cavalcante, L. S., de Andrade, F., Wazlawik, E., Di Pietro, P. F., Maraschin, M., da Silva, E. L. (2011). Mate tea (*Ilex paraguariensis*) improves glycemic and lipid profiles of type 2 diabetes and pre-diabetes individuals: a pilot study. *J Am Coll Nutr.* 30, 320-322.

Kuipers, F., Bloks, V. W., Groen, A. K. (2014) Beyond intestinal soap-bile acids in metabolic control. *Nat Rev Endocrinol*. 10, 488-98

Liao, C.H., Kuo, Y. H., Ho, Y.L., Wang, C. Y., Yang, C. S., Lin, C. W., Chang, Y. S. (2014). Studies on cytotoxic constituents from the leaves of *Elaeagnus oldhamii* Maxim. in non-sall cell lung cancer A549 cells. *Molecules* 19, 9515-9534.

Lo, S. H., Li, Y., Cheng, K. C., Niu, C. S., Cheng, J. T., Niu, H. S. (2017). Ursolic acid activates the TGR5 receptor to enhance GLP-1 secretion in type 1-like diabetic rats. *Naunyn Schmiedebergs Arch Pharmacol*. 390, 1097-1104.

Loizeau, P. A., Barriera, G., Manen, J. F., Broennimann, O. (2005). Towards an understanding of the distribution of *Ilex* L. (Aquifoliaceae) on a world-wide scale. *Biol Skr* 55, 501-520.

Ma, H., Patti M. E. (2014). Bile acids, obesity, and the metabolic syndrome. *Best Pract Res Clin Gastroenterol.* 28, 573–583.

Mendive, J. R. (1940). The occurrence of α-amyrin and ursolic acid in the leaves of *Ilex paraguariensis*, *J. Org. Chem.* 5, 235-237.

Pardau, M. D., Pereira, A. S. P., Apostolides, Z., Serema, J. C., Bester, M. J. (2017). Antioxidant and anti-inflammatory properties of *Ilex guayusa* tea preparations: a comparison to *Camellia sinensis* teas. *Food Funct.* 8, 4601-4610.

Perino, A., Schoonjans, K. (2015) TGR5 and immunometabolism: insights from physiology and pharmacology. *Trends Pharmacol Sci.* 36, 847-857.

Raetsch, C. (2005) The encyclopedia of psychoactive plants. Park Strett Press, Rochester, Vermont, p. 287-290.

Simonsen, J., Ross, W. C. J. (1957). The terpenes. Volume IV, p. 171.

Schultes, R. E., Raffauf, R. E. (1990). The healing forest. Medicinal and toxic plants of the northwest Amazonia. Dioscorides Press, Portland, Ore.

Figure Captions

Figure 1. ^{1}H NMR spectra (400 MHz, $CDCl_3$) of the ethyl acetate fraction obtained from *Ilex paraguariensis* (top) and *Ilex guayusa* (bottom)

Figure 2. Chemical structures of the major triterpenoids of guayusa and maté

Scheme 1. Extraction and isolation procedure of *Ilex paraguariensis* and *Ilex guayusa* leaves. Mass recoveries (as weight percentages) are reported.

Scheme 2. Semisynthetic procedure for preparation of α-amyrin palmitate (**2a**) from a mixture of amyrins (for simplicity only α-amyrins are indicated as starting material)

Highlights:

- Guayusa is growingly popular as a caffeinated beverage, similar to maté
- The phytochemistry and bioactivity of guayusa are still substantially unexplored.
- Guayusa and maté extracts activate TGR5, a transcription factor involved in diabetes
- Ursolic acid is the active constituent from both plants
- The amyrin complex (α/β-amyrin palmitate and palmitoleate) is inactive on TGR5.

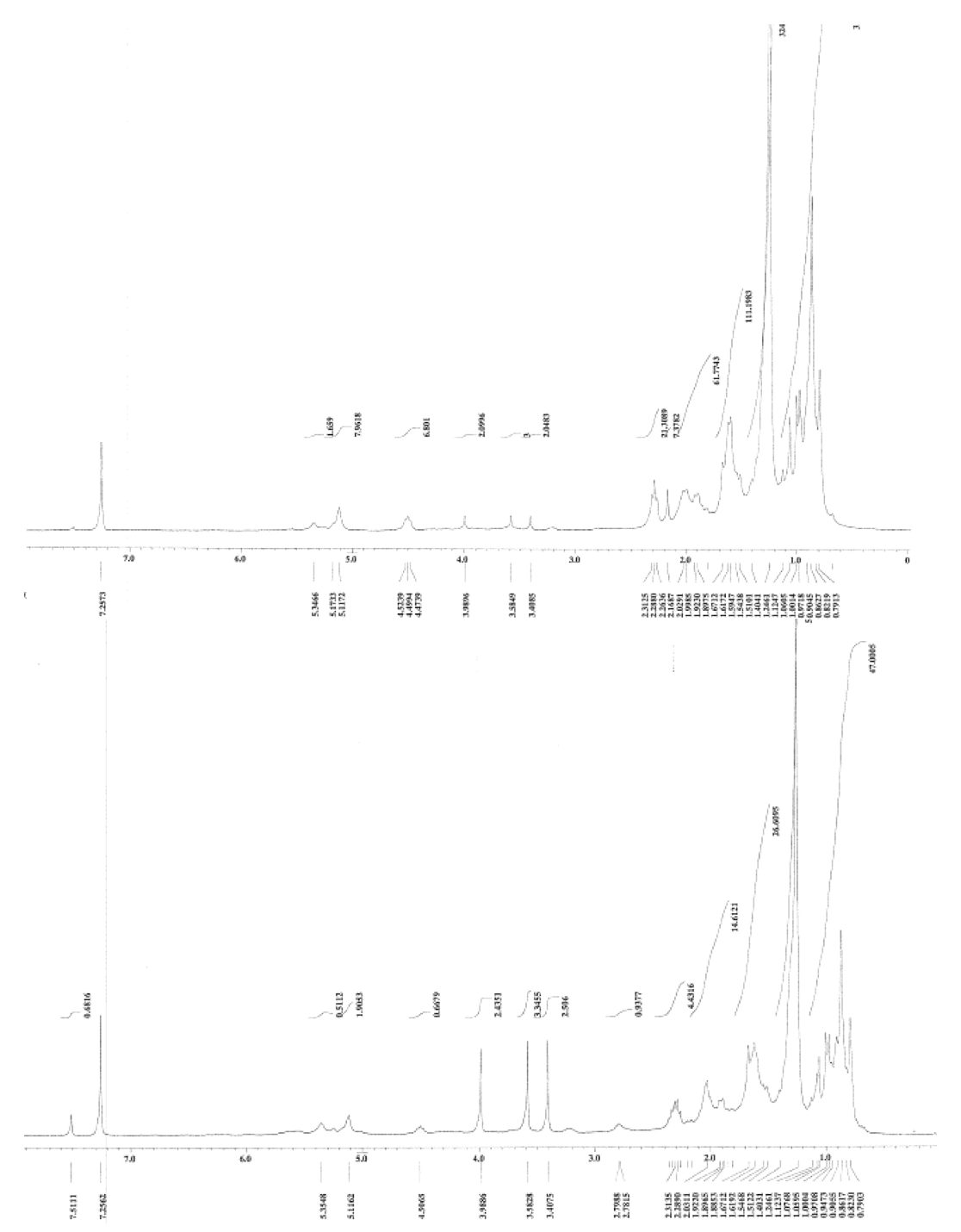
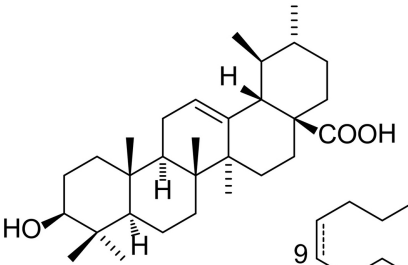
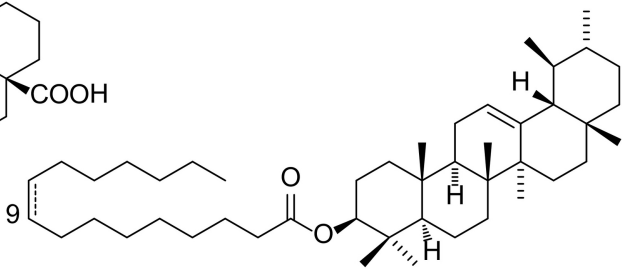


Figure 1

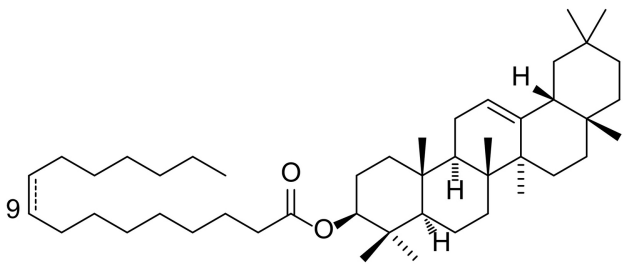


1



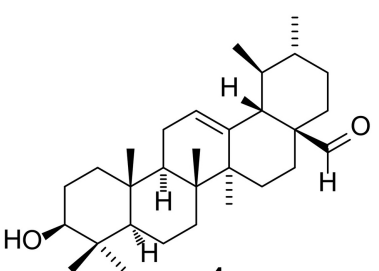
2a

2b Δ^9

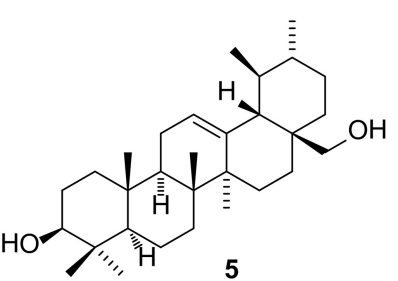


3a

3b Δ^9



4



5

Figure 2